ACTIVITY PATTERNS IN THE ISOLATED CENTRAL NERVOUS SYSTEM OF THE BARNACLE AND THEIR RELATION TO BEHAVIOR [1]

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In the course of working with the central nervous system of barnacles while investigating the mechanism of the shadow reflex (Gwilliam, 1963, 1965), it was noted that most of the nerve trunks displayed persistent rhythmical activity even when the system was completely isolated from any peripheral input. The median photoreceptor could be included in the preparation, but it was not a necessary component of the system that displayed the activity. The fact that barnacle behavior is so markedly periodic (see, *e.g.,* Crisp and Southward, 1961; Southward and Crisp, 1965) leads to the expectation that there may be some demonstrable relationship between the activity of the isolated central nervous system and the behavior of the intact animal.

The idea that centrally determined "programs" serve to direct behavior in the absence of sensory feed-back is now accepted (see Wilson, 1966 for summary). The "command" fibers of the crayfish (Wiersma, 1952; Wiersma and Ikeda, 1964; Kennedy, Selverston, and Remler, 1969 for summary) and certain of the central nervous system cells in the nudibranch *Tritonia* (Willows, 1967; Dorsett, Willows and Hoyle, 1969) are examples of neurons that direct a complex, integrated output event or events. Evidence from the flight system of certain insects (Wilson, 1961) indicates that there is a central oscillator that determines the pattern of motor discharge to the flight muscles, and the rhythmical discharge in the swimmeret motor roots in the crayfish (Ikeda and Wiersma, 1964) is evidence of an oscillator in the crayfish. Indeed, as Bullock (1961, page 51) states: "In principle it should be no surprise to find that a perfectly coordinated sequence of reciprocal activation of antagonistic muscles forming an adaptive action can arise purely centrally."

The work reported here seeks to determine the reality of spontaneous rhythms in the barnacle central nervous system, the patterns of phase relationships in the different nerve trunks, the identity of some of the muscles served by those nerves, and the probable actions of those muscles in terms of the behavior of the intact animal. Another paper will report on the activity of single cells in the central nervous system.

Materials and Methods

The animals used for most of the work were specimens of *Balanus cariosus* (Pallas) collected from the central Oregon coast. Some observations were made on specimens of *B. nubilus* Darwin collected from the same region.

The central nervous system was exposed as previously described (Gwilliam, 1965) and the simple further step of removing it from the animal was easily accomplished. In most preparations the median photoreceptor was included to serve as a test input device to check continuity in certain nerve pathways. Some preparations consisted of the central nervous system removed from the body of the barnacle, but left attached via the great splanchnic nerves to the adductor scutorum muscle. This involved retaining the terga and scuta with the muscle. Such a preparation permitted recording intracellular junctional potentials from the muscle fibers while monitoring the activity in any of the several nerve trunks. These two kinds of activity could be displayed simultaneously so that the temporal relationships could be observed. Such preparations are referred to as "semi-isolated" in the text.

Recording of nerve trunk activity was accomplished with Pt.-Iridium hook electrodes or suction electrodes, amplified through conventional A. C. pre-amplifiers and displayed on a cathode-ray oscilloscope. Muscle junctional potentials were recorded with 3 M KCl-filled glass micropipettes of 10–30 megohms resistance, and were amplified with a neutralized input capacity amplifier. Permanent records were made by photographing the oscilloscope trace with a Grass kymograph camera. Simultaneous two, three, and four channel recording was used as required, and long term activity was occasionally recorded on an ink-writing oscillograph (Grass Model 7 Polygraph).

Recordings were made in an air-conditioned darkroom where the temperature was maintained at 16–19° C which is warmer than the sea water the barnacles usually experience, but colder than temperatures reached on many sunny days when the animals are exposed.

The medium bathing all preparations was "Instant Ocean" artificial sea water (Aquarium Systems, Inc.). This proved superior to either stored natural sea water or barnacle Ringer's solution (Hoyle and Smyth, 1963) as judged by the longevity of preparations exposed to the various media. Under the above conditions preparations could be made to last for up to 36 hours without elaborate precautions, but were seldom used for more than eight hours.

Results

Figure 1 illustrates a ventral view of the central nervous system of *Balanus cariosus* as it is pinned out for recording. Many of the smaller nerves and branches of major nerves have been omitted, the purpose of the diagram being to locate the nerves recorded from and to show the major features of the system. The nerve trunks are named according to Darwin (1854) except where inappropriate (*e.g.,* "median photoreceptor" instead of "ophthalmic ganglion"). Where a specific nerve used was not named by Darwin we have coined a simple descriptive name. Thus, an unpaired nerve originating from the anterior part of the dorsal surface of the ventral ganglion has been identified as the "mid-dorsal" nerve. A pair of nerves from the anterior ventral surface of the same ganglion have been called
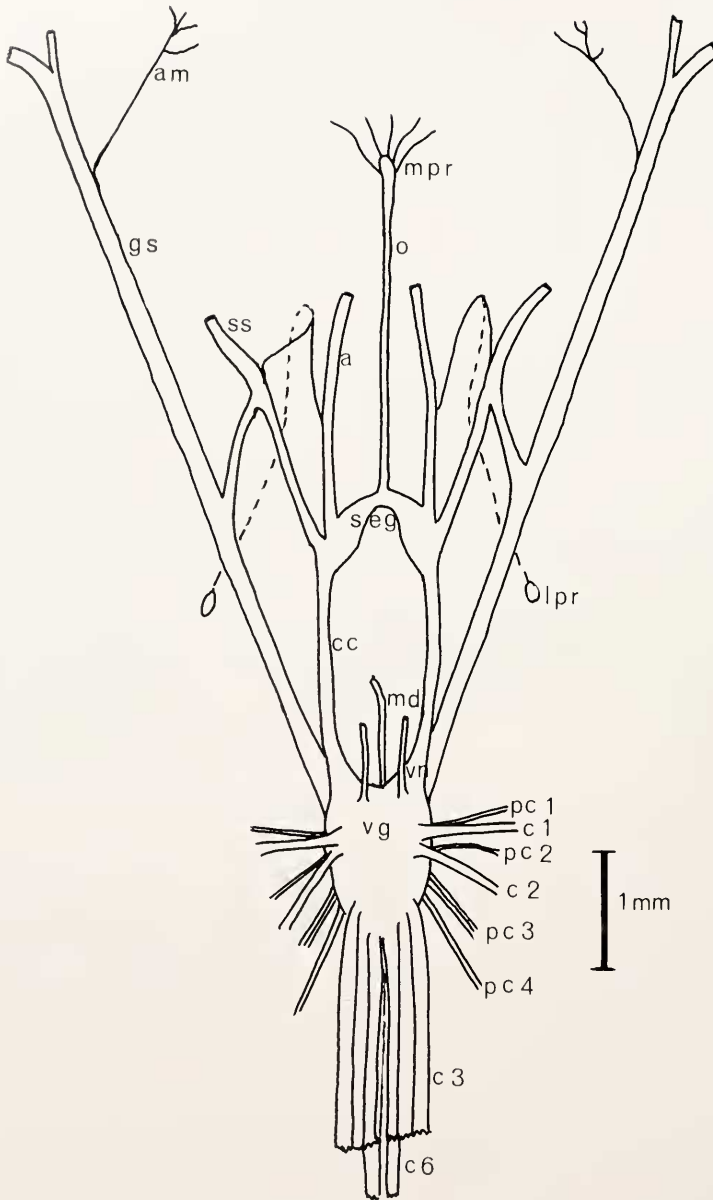
FIGURE 1. Ventral view of the central nervous system of *Balanus cariosus*. The lateral photoreceptors are not included in the preparation used, but are shown for reference. Key to labelling is: am, motor branch to adductor scutorum; a, antennular nerve; cc, circumesophageal connective; c 1–6, cirral nerves; gs, great splanchnic nerve; lpr, lateral photoreceptor; mpr, median photoreceptor; md, mid-dorsal nerve; o, ocellar nerve; pc 1–4, paracirral nerves; seg, supraesophageal ganglion; ss, suprasplanchnic nerve; vg, ventral ganglion; vn, ventral nerves.

simply the "ventral" nerves, and others associated topographically with the main cirral nerves have been called "paracirrals" and numbered in order (Figure 1, pc 1–4). It will be noted that, unlike the situation in those species illustrated by Darwin (1854) and Cornwall (1953), both the first and second cirral nerves are separated from the remainder. Cirrals 3–5 emerge from the ventral ganglion in a group, but the sixth pair are located dorsal to them and are considerably larger, owing in part, no doubt, to the inclusion of the penis nerve in the same sheath.

The connections between the great splanchnic nerve and the suprasplanchnic nerve, and between the suprasplanchnic and the antennular nerve (which, at least proximally, contains the lateral photoreceptor axons) may not always be located exactly as shown, but the general relationships are, in our experience, always as illustrated. Cornwall's interpretation of the innervation of the adductor scutorum muscle as being via his "nerve 4" (the suprasplanchnic of Darwin and Fig. 1) is almost certainly in error. All of the sessile barnacles we have examined have the adductor muscle innervated via the great splanchnic nerve, although the level at which the branch comes off may differ from species to species and indeed, from individual to individual. Further, Cornwall's interpretation of photoreceptor nerves appears to be based in part on Darwin's misconception of the nature of the median photoreceptor (Gwilliam, 1965).
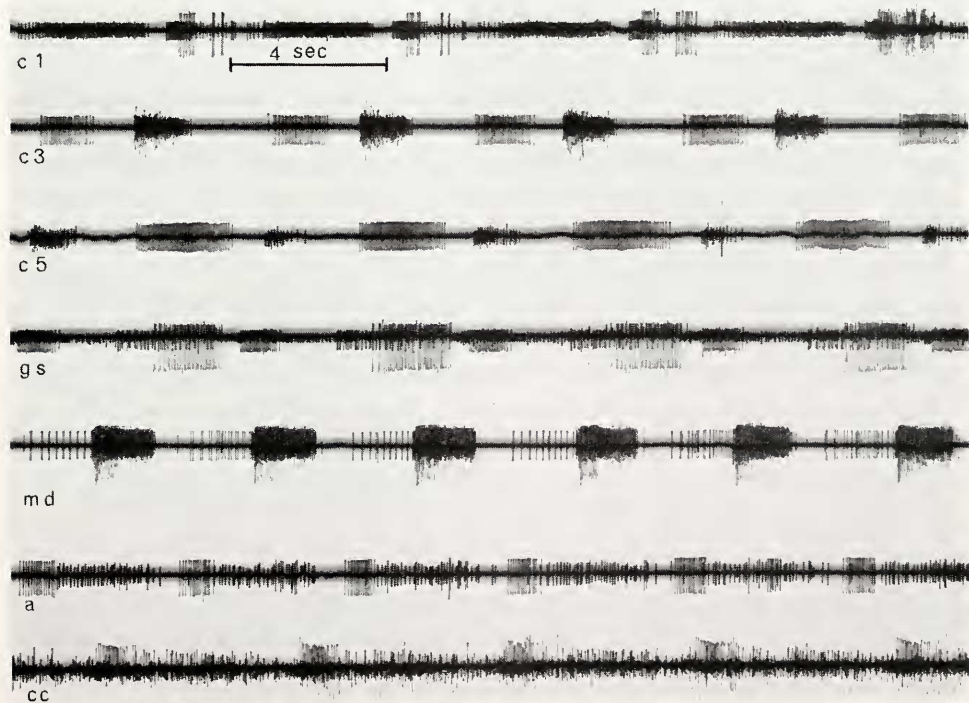

FIGURE 2. Examples of spontaneous rhythmical activity in some nerve trunks of *B. cariosus*. The label at the lower left of each line of recorded activity indicates the nerve trunk from which it was taken. See Figure 1 for identification. Time calibration applies to all records in this figure. Records from several different preparations.

Figure 2 presents segments of filmed records of external recordings from some of these nerves showing spontaneous rhythmical activity in the isolated or semi-isolated central nervous system. Of the major trunks illustrated in Figure 1 only the ocellar nerve and the suprasplanchnic fail to show rhythmical activity. Once the suprasplanchnic is isolated from its connections with the great splanchnic and the antennular, its usual response is complete silence. The ocellar nerve consists principally of sensory axons from the retinular cells but does contain some small efferent fibers (Fahrenbach, 1965) that show no rhythmical activity. All other nerve trunks show rhythms of varying degrees of complexity.

With the possible exception of the first and second (Fig. 2, c 1) all cirral nerves display very similar patterns (Fig. 2, c 3, c 5). The records may appear different from nerve to nerve and preparation to preparation, but almost always consist of a single fiber burst and a multi-fibered burst alternating. The pattern in the first two cirrals is usually more complex and more likely to display random bursts of activity. Sometimes both bursts are prolonged and tend to overlap, but we have never observed them to be completely coincident.

The portion of a record from the great splanchnic nerve (Fig. 2, gs) was taken *en passant* in a semi-isolated preparation, but it is not appreciably different from that seen in an isolated central nervous system. It will be noted that there are at least three different rhythmical fibers, probably a fourth, and a continuous background fiber firing quite regularly. Similarly, the mid-dorsal nerve (Fig. 2, md) has at least three bursting fibers, the patterns of which all overlap.

The antennular nerve (Fig. 2, a) usually displays a rather simple pattern as illustrated but quite often appears to be firing randomly. At other times only the pattern illustrated by the large spikes will be apparent. The circumesophageal connectives (Fig. 2, cc) on the other hand, have quite complex patterns. Usually a major rhythmical burst can be seen, but this is often obscured by much background activity. In this particular illustration the connective was recorded from *en passant*. In cases where the circumesophageal connectives are cut, rhythmical activity is seen only efferent with respect to the ventral ganglion. Isolated supraesophageal ganglia have never, in our experience, shown rhythmical activity of the sort illustrated in Figure 2.

Patterns similar to those seen in isolated and semi-isolated preparations may also be seen in minimally dissected animals. The main difference lies in length of burst and interval between bursts, both of which are usually longer in the more intact animals.

These observations—the consistency and ubiquity of the rhythmical activity in isolated preparations and their presence in minimally dissected animals—suggests to us that the phenomenon is not an artifact of isolation (*cf.* Willows, 1967).

If one accepts the reality of the centrally generated rhythms as a working hypothesis, then one must turn to the normal activities of the intact barnacle to see if there is a correlation between the nervous activity described above and the behavior of the animal. Observations of rhythmical behavior in the intact animal will permit predictions to be made concerning expected patterns in nerves serving particular muscles that can be seen to cause particular movements, and the appropriate recordings made from those nerves.

The most obvious rhythmical behavior a barnacle engages in is "fishing" by

extending the body and cirri to form a net and retracting back into the shell. This process must involve not only some muscles acting sequentially, but because extension of the body is accomplished by a hydrostatic mechanism, there must also be sets of muscles acting 180° out of phase with those muscles involved in retraction. In addition to the normal fishing, Crisp and Southward (1961) identify four other kinds of activity. These have been called testing, pumping, fast beat, and extension. Of these, pumping and fast beat are rhythmical. In all of these activities the basic movements are the same, but they vary in extent and frequency. *Balanus cariosus* displays all of these patterns, except that we have not distinguished clearly between a fast and normal beat. Frequency of beat is variable, but the variability shows no discontinuity that would permit other than a very arbitrary division. It is also the case that *B. cariosus* is not as consistently active as many of the smaller species (Southward and Crisp, 1965). In our experience, normal beat occurs only when the animal is exposed to moving water. Testing, pumping, and extension, however, occur in the absence of sufficient moving water to induce normal beat, and these activities start and stop without obvious stimulation.

Given the kind of behavior described above, and if the central nervous activity is a program which directs that behavior, then there should be a variety of "phase" relationships between the various motor fibers serving the muscles involved in extension and retraction. This can be examined by simultaneous recording from various nerve trunks to see if there is a set of events and sequences that lend themselves to this interpretation. A set of positive observations will not, of course, prove the reality of a central spontaneous program, but it is at least consistent with the hypothesis. This has been done for several nerve trunks, and examples of the results are illustrated in Figure 3.

The records shown in Figure 3 reveal both the in-phase and out-of-phase characteristics of the activity seen in the major nerve trunks. In Figure 3, A, the upper trace is from the mid-dorsal nerve, while the lower trace is from paracirral 4. The
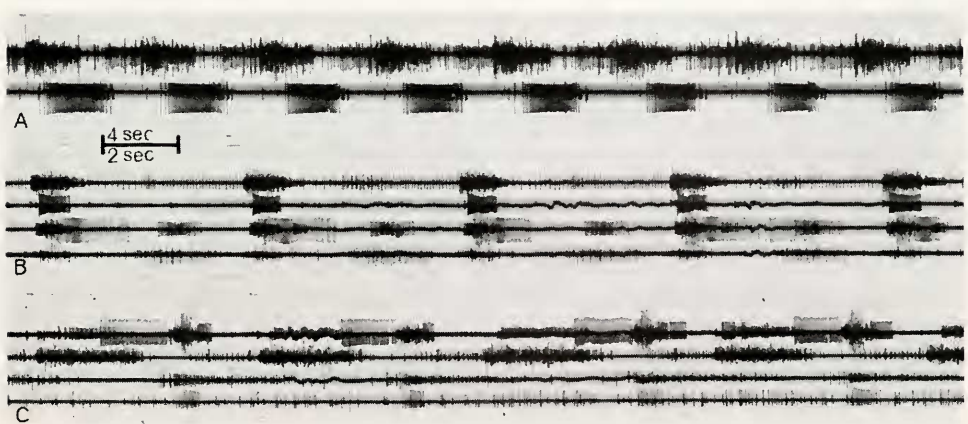

FIGURE 3. Simultaneous recording from various nerves in the isolated central nervous system to show phase relationships; time calibration = four seconds in A, two seconds in B and C; (A.) Top trace, mid-dorsal nerve; bottom trace, paracirral 4; (B.) Top to bottom traces, cirrals 5 and 6; cirral 2; ciral 4; cirral 1; (C.) Top to bottom traces, cirral 4; mid-dorsal; great splanchnic; antennular.

mid-dorsal nerve serves, in part, some of the ventral transverse (dorsal to the nervous system) musculature which exerts pressure on body fluids to bring about an extension of the thorax and cirri. Some fibers from the paracirral nerve serve ventral musculature which is involved in retraction (Gutmann, 1960). In Figure 3, B, all traces are from cirral nerves. It is perhaps significant to note that the principal bursts in two of the nerves serving the three pairs of cirri that make up the actual feeding filter (cirri 4, 5, 6) are just off-set with the posterior cirri leading (lines 3 and 1) as might be expected during a posteriorly originating retraction wave. Cirrals 1 and 2 (lines 4 and 2) have rather different activities in the feeding sequence, and their nerve supply shows somewhat different phase relationships to the others. Care was taken in these recordings to ensure that the timing differences shown could not be due solely to different lengths of conducting pathway based on the assumption that length of nerve from the ganglion was an adequate measure of equal or nearly equal conducting paths. In Figure 3, C, the record from the fourth cirral nerve is from at least four different fibers, and at least three of them are either partially or completely out of phase with the mid-dorsal nerve, while at least one is more or less in phase. A possible explanation of such a record is that the activity coincident and overlapping with activity in the mid-dorsal nerve is from motor fibers activating muscles in the limb base that cause basilar movements during extension. Those out of phase with the mid-dorsal are probably fibers serving the cirral retractor muscles and would be active during withdrawal in the intact animal. The mid-dorsal is out of phase with elements in the great splanchnic and antennular nerves, the latter two showing in-phase bursts. The great splanchnic (Fig. 3, C, line 3) serves (among other things) the adductor muscle and the muscles that depress the mouth cone. These are involved with retraction and closure and would be expected to be out of phase with the mid-dorsal nerves. Similarly, the antennular nerves supply the lateral and rostral scutal depressors. The lateral scutal depressors are concerned, in part, with opening the valves, but the rostrals contract during closure. There is a concerted burst in the antennular in phase with the clearest burst in the great splanchnic, suggesting these are involved in retraction. (The reason for suggesting that the shortest, most clearly defined bursts are associated with the retraction phase is that observations of especially the larger barnacles show quite clearly that extension may be a rather lengthy process, while retraction is usually comparatively rapid.) It is also worth pointing out that muscles involved in increasing hydrostatic pressure and thus causing extension would probably not completely relax. One might therefore expect activity in the nerves serving them to show a regularly varying frequency distribution without ever reaching complete inactivity. Retraction, on the other hand, which is a direct muscular action on the part concerned, might well be discontinuous, or more nearly so. In this connection, discontinuities occur more frequently in the cirral nerves than in the others, and it is the cirri that are served only by retractor muscles (Bullock and Horridge, 1965, page 1181), at least in the distal rami.

Figure 4 presents records that relate some of the activity seen in the isolated nervous system to a behaving motor unit (a single fiber from the adductor scutorum muscle) whose activity could be monitored by observing junctional potentials with intracellular electrodes. The preparation used is that described in Materials and Methods as the semi-isolated preparation. The adductor muscle is very much in-

volved in the rhythmical activity of barnacles in that it is the muscle that closes the valves during the withdrawal-closure reaction.

In Figure 4, A, the upper trace is a recording from the antennular nerve which supplies the rostral scute depressors, while the lower trace is from the adductor muscle. Both these muscles are active during retraction, and the activity in the adductor is virtually coincident with the clearest burst in the antennular. This is what would be predicted if the high frequency burst in the antennular nerve is from the fibers activating the rostral scutal depressor muscles. The secondary burst, of lower frequency, is out of phase with the muscle junctional potentials and may be from those fibers serving the lateral depressors. In Figure 4, B the upper trace is from the great splanchnic nerve, recorded *en passant*. This nerve supplies muscles which are active during extension and retraction. Apart from the adductor muscle and the oral cone depressors, it innervates the large lateral body muscles (numbered 8 by Gutmann, 1960) which are responsible for hauling the body up toward the opercular plates during extension. One would expect, therefore, to find both in-phase and out-of-phase elements in the great splanchnic with reference to adductor contractions, and this is indeed the case.

In the normal behavior sequence the cirri are retracted before the scutes are closed by the adductor muscle. One would expect, therefore, the adductor always to become active toward the end of the cirral burst. Figure 4, C, illustrates the relationship between activity in a cirral nerve (cirral 3) and the adductor scutorum.

Figure 4, D, is from the same sequence as 4, C, but illustrates a neural correlate of a frequently occurring behavioral event observed in intact animals. All barnacle-
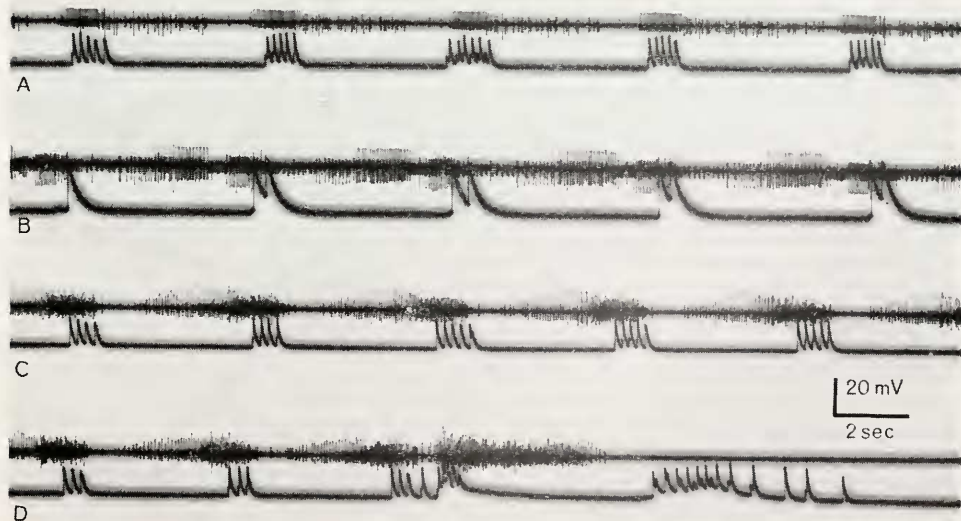

FIGURE 4. Simultaneous recording from various nerves and the adductor muscle (lower trace in all cases). Adductor muscle junctional potentials are D. C. recorded intracellularly. Semi-isolated preparation. Voltage calibration applies to lower trace only; (A.) Antennular nerve, (B.) Great splanchnic nerve (recorded *en passant*), (C.) Cirral 3, (D.) Cirral 3. See text for explanation.

watchers have observed the fact that an undisturbed barnacle will be actively pumping or fishing for a period of time, and then for no obvious reason, undergo withdrawal and closure and remain that way for variable periods. This interruption of activity may itself be rhythmical (Southward and Crisp, 1965) and is commonly observed in most species. For some time we have noted that a particular out-of-rhythm burst of activity, most notable in the cirral and antennular nerves, always preceded a more or less prolonged period of relative inactivity or at least arhythmicity in all of the nerve trunks. Such a burst is seen as the last one in the upper trace in Figure 4, D. It is accompanied by some increased activity in the adductor muscle, but is *followed* by considerable activity and, in this case, an observed massive contraction of the muscle, followed by silence in the cirral nerve. This period of silence and/or arhythmicity lasts for up to five minutes, after which time the characteristic bursting patterns are re-established. Activity very similar to this, but occurring more rapidly, may be induced in such preparations by casting a shadow if the photoreceptor is part of the system.

Observations on many spontaneously active specimens of *B. cariosus* have established that, under laboratory conditions, the beginning of one extension-withdrawal cycle to the beginning of the next one occupies three to seven seconds. Most of the records we have obtained from isolated and semi-isolated preparations of this species show the same periodicity. We have not noted any marked change in periodicity in any of our preparations, but we have not as yet attempted to manipulate environmental factors such as temperature, O_2 concentration, ion balance, or other parameters to see if they have the effect in isolated preparations seen in the intact animals (von Buddenbrock, 1930; Southward and Crisp, 1965).

DISCUSSION

It will be recognized from an examination of the records presented in this paper that the explanations offered for in-phase, out-of-phase relationships and interpretations of sequential events are not rigorously based on a detailed knowledge of the distribution of individual nerve fibers to individual muscles. Recording from multi-fibered bundles that have wide distributions always introduces considerable uncertainty into interpretation. What is clear, however, is that there are consistent phase and sequential relationships and that the interpretations offered are at least consistent with the known distribution of the nerves. It will probably be possible to record from some final motor branches and demonstrate these relationships more conclusively, but to date the adductor muscle has been the only one to lend itself to this procedure.

This particular preparation, when isolated, does not appear to need triggering in any way to initiate or support the patterned activity. In this it is similar to the crayfish abdominal cord as reported by Ikeda and Wiersma (1964), even though a later paper (Wiersma and Ikeda, 1964) demonstrates that pattern maintenance is also achieved by stimulation of command fibers found in the thoracic-abdominal connectives. It is unlike the locust flight system (Wilson, 1961), which requires continued stimulation, and *Tritonia* (Dorsett, Willows, and Hoyle, 1969), which requires some sensory input in the intact animal or electrical stimulation in the isolated central nervous system to trigger the fixed action pattern. Further indication of a truly autogenic system comes from the fact that the rhythmic activity

has been observed to stop and restart some time later completely spontaneously. Cessation of rhythmical activity is preceded by a positive, recognizable event (see Fig. 4) which is itself probably part of the "program." The fact that this aspect of central nervous system behavior corresponds to observed behavior in the intact animal lends credence to the hypothesis that we are indeed observing the neurological basis of behavior in the barnacle.

The preparations described here exclude the possibility that the timing cue for rhythmical activity originates in discharge from peripheral sense organs related to some external event or to the activity of the animal. The possibility that the timing cue is derived from some general level of excitability due to input over sensory fibers cannot be ruled out at this stage, because it is not known what sort of afferent activity is present. The fact that patterned output persists over a period of several hours following isolation, however, argues against such an interpretation.

Behavioral observations on *B. cariosus* indicate that a current of water is needed to initiate fishing. It should be emphasized that the neuromuscular elements and events involved in fishing are the same as those involved in pumping. The difference is in the extent of activity and the frequency with which it occurs. Even in cases where a barnacle is apparently inactive, it is quite conceivable that certain of the same muscles involved in the obvious activities are contracting rhythmically serving a blood circulating function in the absence of a heart (see, *e.g.*, Blatchford, 1970).

Given the present evidence, the best interpretation is that there is a central timing device that must be regarded as "spontaneous" in that it does not depend on externally generated phasic input to determine the periodicity of its output. It is apparent from variations of degree and timing of intact barnacle rhythmical behavior that the output can be altered or modulated. Two possible mechanisms are, a), changes in the immediate environment of the oscillator neurons (*e.g.*, ionic concentration, temperature) as postulated by Mendelson (1971), and b), feed-back from peripheral sense organs, neither of these necessarily operating to the exclusion of the other. There is no direct evidence of the first in the barnacle material, and the second is best demonstrated by the alteration of rhythmical activity seen in the shadow reflex. Work currently going on at University College North Wales, Department of Marine Biology, has established that there are both extension and flexion receptors in barnacle cirri (J. V. Clarke, personal communication), and observations in our laboratory indicate that there is receptor activity fed back to the central nervous system from mechano-receptors associated with the adductor muscle and the opercular muscles. Records from a presumed mechanoreceptor of unknown location have been published (Gwilliam, 1963, Fig. 10). There is as yet, however, no evidence that these receptors modulate on-going rhythmical activity, but it is reasonable to assume they have this effect. It is also apparent that the timer may be turned off, or uncoupled from the rest of the pathway as silent periods in the isolated preparation demonstrate.

It is possible to record from single cells with patterned output in the barnacle ventral ganglionic mass (Gwilliam, 1968) and it is expected that studies of such cells will give more insight into the properties of this particular system.

We wish to acknowledge with thanks a critical reading of the manuscript by Dr. Derek Dorsett which resulted in considerable improvement.

Summary

1. Rhythmical patterns of activity in most nerve trunks of the sessile barnacle, *Balanus cariosus* (Pallas) have been demonstrated to occur in the totally isolated central nervous system at a periodicity consistent with the behavior of the intact animal.

2. When activity in various nerves is compared by simultaneous recording, a pattern of phase relationships is observed that is consistent with the hypothesis that the patterned activity constitutes a program that determines the behavior of the barnacle.

3. The evidence presented suggests that the centrally generated rhythm is autogenic, because in the isolated central nervous system there is no possibility of regular timing cues being made available to central neurons from peripheral sense organs, and no apparent stimulation is required to start and maintain the rhythm.

4. Single muscle fibers in the adductor scutorum muscle attached to the otherwise isolated central nervous system show excitatory junctional potentials with the same temporal rhythm and the expected in-phase, out-of-phase relationships with a variety of nerve trunks including its own supply.

5. It is suggested that observed variation in intact barnacle behavior may be brought about in the system by some direct influence on oscillator neurons and/or sensory feed-back to modulate the extent and timing of rhythmical activity and by uncoupling the timer from the motor output side by turning it off (inhibition) during periods of inactivity. The first of these would also explain frequency variations seen in the isolated preparations in the absence of sensory feed-back.

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